characteristics (CD34, CD3, CD19, NK, DC1and DC2 cell dose). Survival curve analysis by a log rank test revealed that the low DC2 group had a significant risk of developing acute and chronic GVHD (P=0.000 for both). These results suggest that the DC2 count in the peripheral blood on day-28 is a strong predictor for development of GVHD in recipients of PBSC in a matched related allogeneic SCT.

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### A CDNA-BASED ASSAY FOR DONOR-CHIMERISM ANALYSIS OF EPIDERMAL LANGERHANS CELLS

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Early acute GVHD of the skin frequently occurs in patients after allogeneic hematopoietic stem cell transplantation. T cell depletion sufficiently reduces incidence and severity, but does not completely prevent skin GVHD which then leads to a prolonged need for immunosuppressive medication. The activation of donor T cells by residing host antigen presenting cells such as epidermal Langerhans cells (LCs) plays a central role in the initiation of acute GVHD. The absence of donor T cells after depletion delays the switch of LCs from host to donor origin in mice. We and others have provided evidence for a delayed switch in LC chimerism after T cell depleted reduced intensity stem cell transplantation in humans. However, most assays used so far either depend on the detection of the Y-chromosome in skin sections of sex-mismatched transplants. In an attempt to set up a sensitive assay of general applicability, we combined the detection of donor chimerism and tissue specific markers in a single multiplex PCR. We established RT-PCRs for 10 different constitutively expressed genes containing single nucleotide polymorphisms (SNPs) inside their coding regions. These PCRs were combined in a single multiplex PCR and the SNPs were analyzed by the primer extension method (minisequencing) and separated by capillary electrophoresis. We tested this approach on PBMCs of 10 patients and their HLA-matched sibling donors. The assay distinguished all pairs in 1 to 6 out of 10 systems. In a subsequent step, the 10plex PCR was combined with the tissue specific markers langerin for LCs and cytokeratin 10 to distinguish LCs from keratinocytes. The expression of langerin and cytokeratin 10 was detected using gene-specific probes in the same minisequencing reaction used for the detection of SNPs. The resulting 12plex assay distinguished sibling donors from the patients with the same specificity and, in the same reaction, detected Langerin as well as cytokeratin 10 in purified LCs and keratinocytes, respectively. In summary, we established a sensitive assay allowing simultaneous detection of donor chimerism together with the tissue specificity of isolated LCs that is independent of sex-mismatched donors. The addition of further tissue specific markers might allow performing chimerism studies on other tissue resident antigen presenting cells. The use of a cDNA-based assay might also allow combining chimerism analysis with activation- and maturation specific markers in a single assay.

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### SELECTIVE DEPLETION OF ALLOREACTING T CELLS BY TH9402-BASED PHOTODEPLETION AS A TRANSLATIONAL STRATEGY FOR GVHD CONTROL IN HLA-MISMATCHED AND MATCHED DONOR-RECIPIENT PAIRS

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Selective depletion (SD) is a strategy to eliminate host-reactive donor lymphocytes from blood stem cell allografts to prevent GVHD and maintain GVL-effects. We investigated a photodepletion (PD) process, whereby allo-activated donor cells are labeled with a photosensitizing rhodamine-based dye, 4,5-dibromorhodamide 123 (TH9402), and exposed to visible light, which preferentially eliminates allo-activated dye-retaining cells. Stimulator cells were prepared from recipient-derived leukapheresis mononuclear cells (MNCs) and cultured using anti-CD3 and 100 IU IL-2/ml. Responder cells (leukapheresis MNCs) from 3 random HLA-mismatched volunteers and 3 HLA-matched sibling donors were cocultured 1:1 with irradiated stimulators for 3 days. Cultured cells were incubated with 7.5 μM TH9402, followed by dye-extrusion and exposure to 5 Joule light energy in the PD light source (Celmed Bioscience Inc., Canada) at $5\times10^7$ cells/ml in FEP plastic bags. Depletion efficacy was studied by mixed lymphocyte reactions (MLR) in mismatched pairs and by helper-T-lymphocyte precursor (HTLp) frequency assay in matched pairs. All six clinical-scale experiments provided sufficient reduction of allo-reactivity and retention of third party responses as measured against a pool of 5 donors. In mismatched pairs mean reduction of allo-reactivity was 703-fold (± 141) when compared to unmanipulated donors. Third-party responses were maintained, with a mean reduction of only 1.3 ± 0.15-fold. In matched pairs alloreactivity was reduced below the "GVHD-threshold" of 1/100.000 whilst third party responses remained above 1/10.000 precursors. This establishes a clinical scale PD process capable of highly efficient removal of alloreactive lymphocytes from mismatched and matched MLRs while maintaining desirable third party responses. As PD targets activation-based changes in MDR-1 that result in an altered dye efflux, the mechanism of action is distinct from surface-marker-based allodepletion (e.g. CD69, CD25). Thus, PD may overcome instability of activation-based surface marker expression resulting in more consistent and effective depletion. This approach will now be tested in a clinical SD trial.

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### PHARMACOKINETICS (PK) OF MYCOPHENOLATE MOFETIL (MMF) IN PEDIATRIC ALLOGENEIC STEM CELL TRANSPLANT (ALLOSCT) RECIPIENTS

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MMF PK in children may impact on the incidence of moderate/severe acute GVHD following AlloSCT (Osunkwo/Cairo et al, BBMT 2004). The objective of this study is to evaluate effects of age on the PK of MMF in pediatric AlloSCT recipients. From 1/04 – 8/06 we enrolled 31 pediatric AlloSCT with 27 being evaluable: mean age 8.5 yrs; wt 34.3 kg; M:F=12:15; NBL PR (n=3), SCD (n=3), AML (CR1 [n=4], CR2 [n=1], CR3 [n=1], relapsed/induction failure [n=3]), SAA (n=5), CML CP (n=1), ALL (CR1 [n=1], CR2 [n=2], CR3 [n=1]), HD CR2 (n=1), ALCL refractory (n=1); donor sources: MFD (6/6 PBSC [n=7], 6/6 BM [n=3], 5/6 PBSC [n=3]), 6/6 related CB (n=1), UCB (6/6 [n=2], 5/6 [n=3], 4/6 [n=7]), and 8/10 MUD PBSC (n=1). Cohort 1 [< 6 yrs] (n=8); 2 [6-12 yrs of age] (n=9); 3 [12–16 yrs] (n=10). GVHD prophylaxis included tacrolimus (on Day –1 or 1st day of conditioning to maintain concentrations 5-20 ng/mL) and MMF (900 mg/m$^2$ IV Q6H starting on Day +1, then converted to PO [same dose] after Day +14). Serum samples for MPA were drawn on Day +1, +7, and +14 at hour 0, 0.5, 1, 2, 3, 4, and 6 post-dose. MPA plasma concentrations were determined by reverse-phase HPLC. MMF dose was adjusted to maintain MPA trough 1-3.5 mg/L. The mean CD34$^+$ cell dose/kg = $27.5\times10^5$, TNC dose/kg = $50.3\times10^7$. Time to neutrophil (ANC ≥ 500/mm$^3$ x 2 d) and platelet engraftment (untransfused count ≥ 20K x 7 d) was 22 d and 36 d, respectively. Mean f/u was 448 d. Mean MPA PK on Day +14: Cmax=17.6 mg/L, Tmax=1.82 h, total MPA trough=0.85 mg/L, $AUC_{0\text{-}6}$=39.4 mg·hr/L, $T_{1/2}$=1.3 h, Vd=1.5 L/kg, and CL=1.2 L/kg/h at a mean MMF dose of 1056 mg/m$^2$ IV Q6H. Age cohorts are shown in Table 1. Incidence of GI adverse events attributable to MMF was 59% (nausea/vomiting [n=13], diarrhea [n=8], abdominal pain [n=3], pneumatosis intestinalis [n=1], gastritis/colitis [n=2]). Kaplan-Meier probability of grade II-IV aGVHD following related [n=14] and unrelated [n=13] donors was 60.8% (15/27 evaluable pts), cGVHD was 25.2% (5/22 evaluable pts) and 1 year OS was 64.4% (CI: 45.6-83.1%). In comparison to MMF PK in adult AlloSCT pts